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Review

The quinone-binding and catalytic site of complex II

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ABSTRACT

The complex II family of proteins includes succinate:quinone oxidoreductase (SQR) and quinol:fumarate oxidoreductase (QFR). In the facultative bacterium *Escherichia coli* both are expressed as part of the aerobic (SQR) and anaerobic (QFR) respiratory chains. SQR from *E. coli* is homologous to mitochondrial complex II and has proven to be an excellent model system for structure/function studies of the enzyme. Both SQR and QFR from *E. coli* are tetrameric membrane-bound enzymes that couple succinate/fumarate interconversion with quinone/quinol reduction/oxidation. Both enzymes are capable of binding either ubiquinone or menaquinone, however, they have adopted different quinone binding sites where catalytic reactions with quinones occur. A comparison of the structures of the quinone binding sites in SQR and QFR reveals how the enzymes have adapted in order to accommodate both benzo- and napthoquinones. A combination of structural, computational, and kinetic studies of members of the complex II family of enzymes has revealed that the catalytic quinone adopts different positions in the quinone-binding pocket. These data suggest that movement of the quinone within the quinone-binding pocket is essential for catalysis.

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1. Introduction

Members of the complex II family of enzymes are membrane-bound protein complexes that couple the interconversion of succinate and fumarate with the reduction of quinone or oxidation of quinol [1–4]. Primary members of the complex II family of proteins include succinate:quinone oxidoreductase (SQR) and quinol:fumarate oxidoreductase (QFR) [1–3]. SQR, the only membrane-bound component of the Krebs' cycle, catalyzes the oxidation of succinate to fumarate during aerobic respiration, coupled with the reduction of quinone to quinol within the membrane domain. SQR is present in the inner mitochondrial membrane and plasma membrane of many aerobic and facultative bacteria such as *Escherichia coli*. QFR is found in anaerobic or facultative bacteria and lower eukaryotes, where it couples the oxidation of a variety of quinols with the reduction of fumarate as part of an anaerobic respiratory chain [1–4]. Complex II enzymes are usually composed of four distinct subunits with two encompassing a hydrophilic domain attached to two membrane integral hydrophobic subunits. There are exceptions, however, where the hydrophobic subunits have fused to form a single transmembrane subunit [2–4] or in the case of parasitic protists where up to six hydrophobic subunits are present [5].

In this article we will focus on the prototypical complex IIs which contain four subunits such as those found in the inner mitochondrial membrane of mammals and in the facultative bacterium *E. coli* [1,3]. The overall structure of SQR (or *E. coli* QFR) (Fig. 1) is a hydrophilic flavoprotein subunit (SdhA for SQR or FrdA for QFR) containing a covalently bound FAD cofactor and the dicarboxylate-binding site interacting with an iron-sulfur protein subunit (SdhB, FrdB). The iron–sulfur protein subunit contains three distinct Fe–S clusters ([2Fe–2S], [4Fe–4S], [3Fe–4S]) arranged to form a linear electron transport chain between the FAD cofactor to the quinone-binding site at the interface of the membrane domain. At the interface between the two hydrophobic membrane spanning (SdhC and SdhD or FrdC and FrdD) subunits and the Fe–S protein lies the catalytic quinone-binding site where quinone reduction/quinol oxidation occurs. In the case of mammalian and *E. coli* SQR the SdhC and SdhD subunits also provide the histidyl axial ligands for the low spin heme *b* present in complex II [6,7]. QFR from *E. coli* is atypical for complex II family members in that it lacks heme even though it is fully capable of catalyzing the same reactions with quinones and/or dicarboxylates as heme containing complex IIs [8–10]. It is of interest therefore, that recent mutational studies of *E. coli* and yeast SQR have shown that the heme appears dispensable for reaction with quinones within the membrane environment although it does provide structural stability to the enzyme [11,12].

The X-ray structures for the *E. coli* SQR [6] and QFR [8] were the first obtained for the respective members of the complex II family, however, these structures were rapidly followed by high resolution structures from the bacterium *Wolinella succinogenes* for QFR [13], and mitochondrial SQRs from pig [7] and chicken [14,15]. The combination of structures showed that the SdhA and SdhB subunits from all

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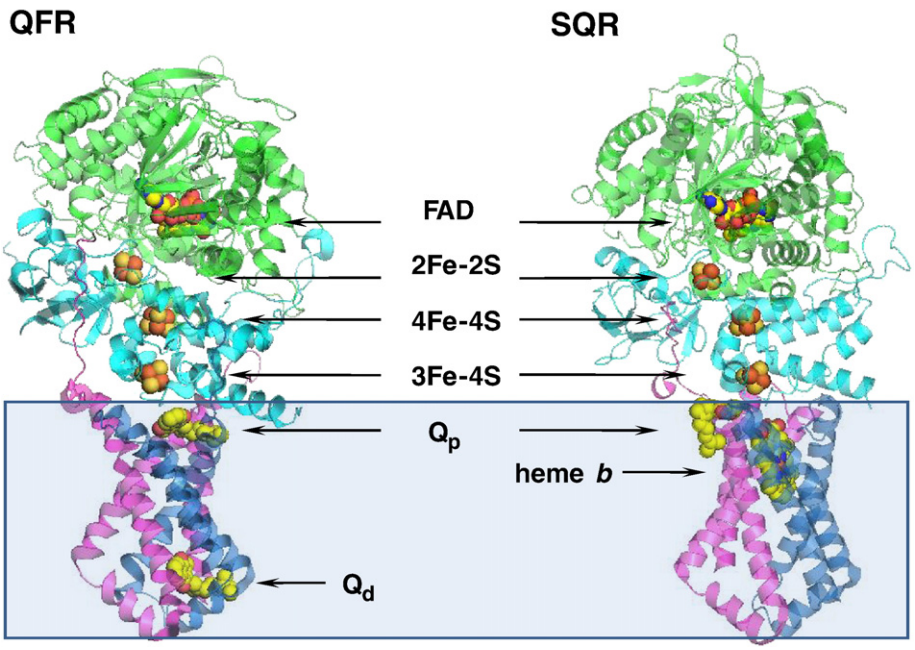

Fig. 1. Structure of *E. coli* QFR and SQR. Left side: Shown is the overall structure of *E. coli* QFR (pdb 1LOV). The FrdA flavoprotein subunit is shown in green, the FrdB Fe–S protein subunit is shown in cyan, the FrdC transmembrane anchor protein is shown in magenta, the FrdD transmembrane anchor protein is shown in blue. The FAD, [2Fe–2S], [4Fe–4S], [3Fe–4S] redox centers are shown as red and yellow spheres. At the bottom the bluish area indicates the membrane domain. The two quinone molecules in QFR, Q_P and Q_d are shown as yellow spheres. Right side: Shows the overall structure of *E. coli* SQR (pdb 1NEK). The subunit and redox center coloring is the same as for the QFR molecule shown on the left. The heme *b* is shown as yellow spheres near the quinone-binding site of SQR.

organisms, including the FrdA and FrdB subunits from both *E. coli* and *W. succinogenes* QFR were highly similar. There were differences, however, in the structures of the transmembrane SdhC and SdhD subunits compared to their QFR counterparts. It was found, however, that amino acid residues that appear to be essential for interaction with quinone were absolutely conserved in *E. coli* and the mitochondrial SQRs [6,7,15]. This makes *E. coli* SQR an excellent model system for structure/function studies of complex II because of the high levels of protein expression and the ease of genetic manipulation of the bacterial enzyme.

A number of heterozygous mutations in the human *SDHB*, *SDHC*, and *SDHD* genes which cause pheochromocytomas and paraganglioma tumor formation have been described (reviewed in [16]). Recently a new gene termed SDH5 which is an assembly factor required for the covalent-flavination of SdhA has also been shown to be associated with tumor formation [17] although the protein product of this gene is not assembled into the mature complex II. Nevertheless, the majority of mutations associated with tumor formation in humans are found in the SdhB, SdhC, and SdhD proteins whose proper assembly is necessary to form the quinone-binding site of complex II [1–3]. Therefore, characterization of the quinone-binding site is important to our understanding of how complex II contributes to disease in different human tissues. Below we discuss and compare the quinone-binding sites of *E. coli* SQR with its mitochondrial counterparts and also with *E. coli* QFR.

2. Distal quinone binding site (Q_D) of *E. coli* QFR and pig SQR

Before any X-ray structures for complex II had become available evidence for the heterogeneity of complex II bound ubisemiquinones in the bovine enzyme was obtained by EPR [18,19]. These data suggested that the two benzoquinone rings of ubiquinone (UQ) were only 7.7 Å apart and perpendicular to the membrane plane [19,20]. Therefore, it was of interest that the initial publication of a structure for any member of the complex II family was that for *E. coli* QFR at 3.3 Å [8] and in this structure two menaquinone (MQ) molecules on opposite sides of the membrane were observed (Fig. 1, left side). The menaquinone molecule near the periplasmic side of the membrane-spanning FrdC and FrdD subunits was termed Q_D (for distal side). Although the arrangement of two quinones on opposite sides of the membrane-spanning region is reminiscent of the cytochrome bc_1 [21,22] and photosynthetic reaction center [23,24] it was difficult to understand how the Q_D quinone could be involved in electron transfer because of the large distance (~27 Å) from its nearest redox center the quinone proximal to the [3Fe–4S] cluster (Q_P) [1,3,8]. The presence of the Q_D-site on the opposite side of the membrane was also not consistent with the spatial proximity of the two ubisemiquinones suggested to be present in bovine mitochondrial membranes [18,19]. It is generally believed that nature has selected electron-tunneling reactions in proteins to occur over a rather short range of 4–14 Å [25]. The X-ray structure of *E. coli* QFR did not show any redox center between the two menaquinone molecules that was capable of mediating electron transfer [8] thus the role of the quinone at the Q_D site was unclear. Mutational studies done with the *E. coli* QFR [10] and yeast [26] and homology models [27] also suggested the potential for two quinone binding sites. It was interest, therefore, when the first eukaryotic SQR structure (pig) was determined [7] that two molecules of the quinone-binding site inhibitor 2-thenoyltrifluoroacetone (TTFA) were observed on opposite sides of the membrane-spanning subunits in a 3.5 Å structure (pdb code 1ZPO) similar to the observed menaquinone molecules in *E. coli* QFR. Although these data [7,8] would seem to support the existence of two quinone binding sites in members of the complex II family of proteins they must be taken with caution. First, there was stronger electron density for the TTFA molecule at the site proximal to the [3Fe–4S] cluster than that for the second TTFA molecule at the site termed Q_D [7]. It was also noted that the primary contacts for the TTFA molecule at Q_D were mainly through two water molecules rather than through the protein backbone and it was suggested that the binding of TTFA to this distal site was much weaker than that at the proximal site [7]. Also the second TTFA molecule in the pig SQR is 15 Å from the edge of the heme *b* which as noted above is not in the range for productive electron transfer reactions [7,25]. It could be argued that conformational changes in the SQR could move the Q_D site closer to the heme

facilitating electron transfer, however, it is clear from studies of yeast and *E. coli* SQR that the single heme *b* is not essential for electron transfer [11,12]. Second, a more recent structure of *E. coli* QFR at 2.7 Å in the presence of the quinone-site inhibitor 2-heptyl-4-hydroxy-quinoline-*N*-oxide (HQNO) showed only a single HQNO molecule at the Q_P-site, whereas, the site at Q_D was empty and filled with diffuse density that probably was from a detergent or phospholipid molecule [28]. Third, the highest resolution complex II structures currently available (2.1 Å, pdb code 2FBW) from avian sources shows only a single inhibitor present which is always at the quinone-binding site proximal to the [3Fe–4S] cluster [15]. Fourth, *E. coli* SQR structures also show only a single quinone-binding site even when crystallized under a variety of conditions and with different inhibitors present [6,29,30]. Fifth, extensive kinetic studies of both *E. coli* and bovine SQR and QFR [3,9] are consistent with a single functional quinone-catalytic site in the enzymes. It can now be suggested that the interpretation of the kinetic data pointing towards the presence of the second quinone-binding site may be due to the use of membrane-bound protein complexes and assay systems that are based on primary (quinone) and secondary electron acceptors (DCIP, MTT) used in the assays [10,26]. It should also be noted, that in both the *E. coli* QFR [10] and yeast [26] systems mutational analysis of the putative second quinone-binding site showed that significant quinone-reductase activity remained in the mutants suggesting that long range structural alterations in the protein could account for the loss of activity. These findings along with the long electron transfer distances from the primary electron transfer chain of complex II suggest that the distal quinone binding site seen in complex II enzymes may result from non-specific binding of inhibitors used at high concentrations during the crystallization procedure.

It should be noted that the functional quinone-binding site of the di-heme QFR from *W. succinogenes* [31,32] and from the di-heme SQR from *Bacillus subtilis* [33] is on the periplasmic side of the membrane. The di-heme members of the complex II family do not have the problem of the spatial distance between redox centers to allow productive electron transfer to occur across the membrane domain. The finding that the protonation/deprotonation of quinone occurs on the positive side of the membrane (i.e., periplasmic space of gram negative bacteria) also helps explain the coupling activity of the di-heme SQR/QFR to the transmembrane electrochemical potential [2,31–35] which does not occur in the prototypical *E. coli SQR*/QFR or mammalian SQR.

The existence of the distal cavity capable of binding quinones may indicate that remnants of an evolutionarily conserved second quinone-binding site is present in complex II enzymes from a variety of organisms [36], however, it would appear that there is only a single functional quinone binding pocket that is proximal to the [3Fe–4S] cluster in single heme *b* containing complex II enzymes, as seen in *E. coli* and avian SQR and more recent QFR structures. The weak binding of the Q-site inhibitor TTFA seen in the SQR pig structure (1ZPO) may reflect the high concentration of inhibitor used during crystallization and the evolutionary remnant of a Q-binding site in mammalian complex II.

3. Common properties of the functional Q-sites of SQR and QFR

There are several similar features that define the quinone-binding site in both SQR and QFR that are discussed below. It should be noted that one difference is that *E. coli* QFR is atypical of complex II family members in that it does not contain or require a *b*-type heme for catalysis or its assembly although it is quite proficient in catalysis with both benzo- and napthoquinones [9]. In *E. coli* QFR the functional quinone-binding cavity (Fig. 2A) occupies a position near the cytoplasmic side of the membrane and involves amino acid residues from the FrdB, FrdC, and FrdD polypeptides. The [3Fe–4S] center is 7 Å (edge-to-edge) from the quinone binding site and separated by a short loop between Cys204 and Cys210 of FrdB [8,28]. The best current resolution for *E. coli* QFR structures is at 2.7 Å, therefore waters have not been specifically placed in structures of the quinone-binding site. Structures with both menaquinone and its analogue HQNO show that they occupy similar positions in the quinone-binding pocket and both carbonyl oxygens of the napthoquinone establish H-bonding to the protein.

Similar to the functional quinone-binding site in QFR the SQR binding site is formed by a pocket near the cytoplasmic interface formed by amino acid residues from the SdhB, SdhC, and SdhD subunits (Fig. 2B). One difference is the close proximity of the heme *b* propionate to the site where quinone binds in SQRs from *E. coli*, mammalian, and avian sources [6,7,15]. The *E. coli* SQR X-ray structure has been determined with bound UQ (2.7 Å), and the quinone inhibitor analogues, Atpenin A5 (2.6 Å), pentachlorophenol (PCP, 3.2 Å), DNP-17 (3.2 Å), carboxin (2.4 Å), and an empty UQ-binding site (3.2 Å) [6,29,30]. Structures of mammalian (pig) complex II with UQ (2.5 Å) and the inhibitor TTFA (3.2 Å) [7] and chicken SQR with UQ (2.4 Å), carboxin (2.1 Å), and an empty site [14,15] have also been published. In all SQR structures from whatever source the same amino acids function in binding of ubiquinone or inhibitors showing the high degree of homology for the functional quinone-binding site from prokaryotes to eukaryotes.

3.1. Interaction of quinones with catalytic sites in complex II

The chemical numbering for substitutions of benzo- and napthoquinone rings used in the discussion is as shown in Fig. 3. The numbering used reflects the position of the isoprenoid tail to identify each face of the quinone ring for UQ (2, 3-dimethoxy-5-methyl-6-isoprenyl-1,4-benzoquinone), and MQ (5-methyl-6-isoprenyl-1,4-napthoquinone).

Ubiquinone and MQ both show asymmetrical binding with a stronger binding for the O1 atom compared to the O4 carbonyl oxygen atom [6,28,37]. In both SQR and QFR there are two H-bonds to the O1 carbonyl oxygen atom with one amino acid from the Fe–S subunit providing one bond and the other coming from an amino acid in the transmembrane domain subunits. Each quinone-docking site consists of a Trp (SdhB Trp164, FrdD Trp14) residue and a second amino acid that normally has a high pK_a in its free form (SdhD Tyr83, FrdB Lys228). The Trp residue is found on the same plane of the face of the quinone ring for either UQ or MQ [6–8,14,15,28,30]. One difference at the primary sequence level is that in one case the Trp residue comes from the hydrophobic domain subunit FrdD, whereas, in the other it comes from the Fe–S protein SdhB but in the three dimensional structure the Trp adopts the same conformation over the quinone ring. There are additional hydrogen bond interactions between FrdB Lys228, with Gln B225 and Cys B204; and SdhD Tyr83 with SdhC Arg31 which likely decrease the pK_a of these potential H-exchangeable groups. Substitution of either SdhD Tyr83 or FrdB Lys228 has severe effect on quinone-binding and catalytic activity, however, the remaining Trp residue in each site was still sufficient for residual quinone-binding [37–39]. In QFR FrdD Trp14 appears to be important to the site by creating a hydrophobic wall and by providing interaction to the first two isoprenoid units of MQ consistent with the K_m for UQ and MQ being an order of magnitude lower for quinones with two isoprenoid units rather than one [9,37].

On the other side of the quinone molecule where H^+ exchange can occur (the O1 carbonyl oxygen atom) FrdC Glu29 and SdhC Ser27 are suggested as sites for proton acceptor/donors [8,29,37]. In the QFR structure [8,28] FrdC Glu29 is clearly within H-bond distance to the O1 carbonyl oxygen of MQ, whereas, in the original SQR structure [6], SdhC Ser27 is about 4.5 Å from the O4 carbonyl of UQ. Site-directed substitution of these residues shows no effect on ligand binding which is consistent with strong asymmetric Q-binding via the O1 carbonyl. Additional confirmation of the *E. coli* QFR quinone-binding site was

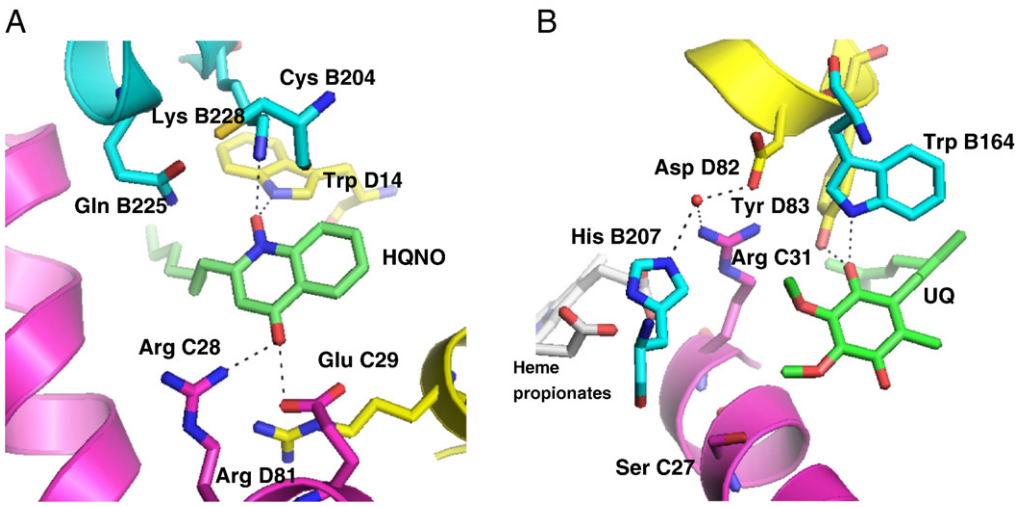

Fig. 2. Functional quinone-binding site of QFR (A) and SQR (B). (A) View of the Q-site of *E. coli* QFR with the menasemiquinone analogue HQNO bound. The structure is drawn from pdb file 1KF6 (2.7 Å). The HQNO is shown in green with the dashed lines to the O1 oxygen atom (which would be present in menaquinone) indicating H-bonds from Lys B228 and Trp D14. Cys B204, Gln B225, and Lys B228 are shown in cyan, and Trp D14 in yellow. On the other side of the binding pocket Arg C28, Glu C29 and Arg D81 are shown with the dashed lines indicating H-bonds to the O4 oxygen atom. Arg C28 and Glu C29 are shown in magenta, and Arg D81 in yellow. (B) View of the Q-binding site of *E. coli* SQR with UQ bound at the entry of the binding pocket. The structure is obtained from pdb file 1NEK (2.5 Å). The UQ molecule is shown in green with the dashed lines indicating H-bonds from Trp B164 and Tyr D83 to the O1 oxygen atom of UQ. Trp D83 is shown in yellow and Trp B164 in cyan. Also shown are His B207 (cyan), Arg C31 (pink), and Asp D82 (yellow) which form H-bonds to a conserved water molecule shown as a red sphere. Shown at the bottom of the figure is Ser C27 (magenta) which forms an H-bond to the O4 oxygen atom once the UQ has moved into the catalytic position (not shown). In the catalytic position His B207 also forms a bond to the 3-methoxy group of the benzoquinone ring. In white and red to the left are shown one of the heme propionates which are near the quinone-binding pocket in SQR.

obtained by electrochemical Fourier-transform infrared (FTIR) analysis. FTIR data showed that MQ carbonyl vibrational modes were downshifted upon MQ binding to isolated QFR which is indicative for strong hydrogen bonds to both menaquinol carbonyl groups with the protein [37]. The observed signal was, however, split reflecting a weaker binding of one of the C = O groups. The stronger binding was

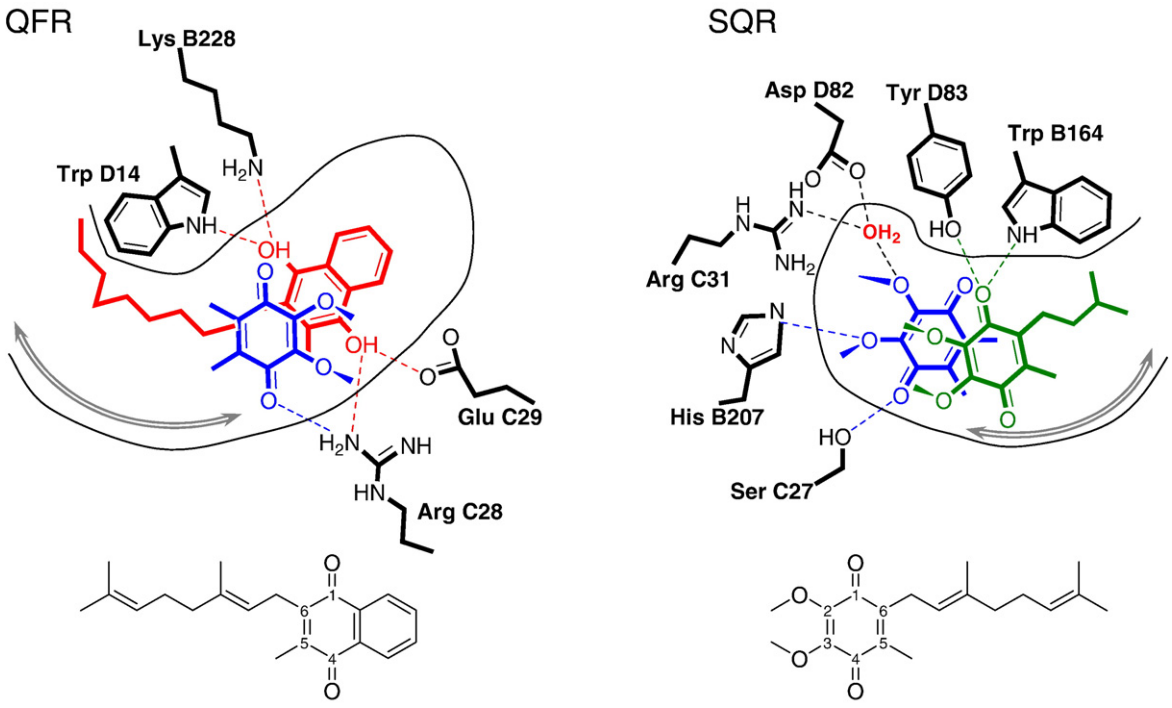

Fig. 3. Model of the movement of quinones in the quinone-binding site of QFR and SQR. (Left side) On the left is shown the model of the movement of MQ (red) and UQ (blue) in the quinone catalytic site of *E. coli* QFR. At the bottom of the figure on the left is shown an MQ molecule including the numbering systems used in the text indicating the isoprenoid side chain at the six positions and the carbonyl positions 1 and 4. In the model it is shown that the C1 carbonyl of MQ forms strong H-bonds with Lys B228 and Trp D14 and the C4 carbonyl forms bonds to Glu C29 and Arg C28. When UQ is present a similar arrangement of H-bonds forms to the C1 carbonyl of UQ, however, a pendulum like motion causes UQ to swing into a position where the C4 carbonyl forms a bond with Arg C28 and not with Glu C29 which only bonds to MQ. The gray arrow indicates the motion of the quinone. (Right side) The motion of UQ within the quinone-binding pocket of SQR. At the bottom is a UQ molecule indicating the numbering systems used in the discussion to note the carbonyl and methoxy groups of the benzoquinone ring. As discussed in the text the figure shows in green UQ docking to the entry site of the quinone binding pocket where the C1 carbonyl forms H-bonds to Trp B164 and Tyr D83, however, there is no nearby residue to H-bond to the C4 carbonyl. Upon delivery of electrons from the [3Fe–4S] cluster a conformational pendulum like movement occurs with the UQ moving to the catalytic position shown in blue. An H-bond with Ser C27 is formed to the C4 carbonyl and there are also interactions with His B207 to the 3-methoxy group and through a water molecule (shown in red) to Arg C31 and Asp D82 to the 2-methoxy group. The gray arrow indicates the pendulum like motion of the quinone within the binding pocket.

clearly attributed to the O1 carbonyl in both QFR and SQR [37]. These data are consistent with effects observed on binding of quinone analogues. In QFR it was found that there was a 100-fold increase in the K_d for HQNO binding in the FrdB Lys228Leu consistent with the Lys residue acting as the normal ligand for the O1 carbonyl [37].

Using the *E. coli* SQR enzyme as the model [6] it is found that side chains of SdhB Trp164 and SdhD Tyr83 are direct ligands of the O1 atom of UQ. SdhD Tyr83 forms and additional H-bond with the side chain of SdhC Arg31 which may lower the pK_a of Tyr83 facilitating its ability to act as a proton donor to UQ [6]. Other residues absolutely conserved and in the vicinity of the O1 atom of UQ in the SQR family are polar residues SdhC Arg31 and SdhD Asp82. Site directed mutants were constructed for both the Arg31 (R31L) and Asp82 (D82L) residues and it was found that there was a very severe affect on the enzymes ability to reduce UQ [38]. Conversely there was somewhat disparate results obtained when SdhD Tyr83 was mutated in *E. coli* SQR [37] versus when the equivalent residue was changed in yeast SQR [39]. The X-ray structures of SQR [6,7,14] show the Tyr residue H-bonded to the O1 atom of UQ and when the Tyr was mutated in the yeast enzyme an almost total loss of the enzymes ability to reduce quinone was found. This is consistent with the Tyr residue being a direct proton donor to the quinone [39]. However, it was found that site-directed mutants of *E. coli* SdhD Tyr83 retained 15–28% of wild-type quinone reductase activity suggesting that some other residue or a water molecule may act as the direct proton donor to UQ [38]. In subsequent unpublished studies using purified isolated SQR we have found that the Tyr83Phe mutation does have a significant (~28-fold) increase in the K_m for binding of UQ. The data in both yeast and *E. coli* SQR are in agreement that Tyr83 plays an important role in the function of the quinone-binding site although it may be that another residue or water molecule may substitute as a direct proton donor to the O1 atom of UQ in mutant forms of the enzyme. The differences between the yeast and *E. coli* SQR may reflect the different ubiquinone analogues used for measurement of quinone-reductase activity [38,39] or subtle differences in the architecture of the quinone binding site in the two organisms.

In the QFR FrdC Glu29Leu mutant [37] there is a significant rise in the pK_a of the menaquinol oxidase reaction supporting the role in wild-type QFR that Glu29 is an H^+-accepting residue [40]. In the wild-type QFR structure with HQNO [28] the O4 carbonyl of the inhibitor, in addition to forming an H-bond to Glu29, is also 3.2 Å from FrdC Arg28 which either directly or via a water molecule may act as a proton acceptor in the mutant enzymes that show a higher pK_a for the menaquinol oxidase reaction. In these same mutants, however, where menaquinol oxidation is altered the kinetic parameters for quinone reduction are similar to those of wild-type QFR [37]. There are several explanations for this observation. First, lack of an effect of the Glu29Leu substitution on UQ reduction is consistent with the suggestion that FrdC Arg28 may directly, or via an H-bonded water molecule, participate in MQH_2 oxidation and define the high pK_a of the reaction. Thus, Arg28 may act as a proton donor for UQ, in the mutant suggesting that this partial reaction is not rate limiting for UQ reduction. Alternatively, one may suggest that Glu29 may not be the direct proton donor for UQ, but rather interacts with the 3-methoxy group of the ubiquinone ring. This would result in a 3–4 Å shift in the position of the UQ ring towards the entry of the Q-binding site that would establish an H-bond between the O4 atom and Arg28 while keeping the O1 atom coordination. This explanation is consistent with the QFR structure with the UQ-analogue inhibitor (DNP-19) that shows the predicted shift in its ring position [8]. These findings also point out that it would be beneficial if X-ray structures were available with UQ bound in wild-type QFR or the FrdC Glu29Leu mutant enzyme. In summary, a distinct feature of the Q-site in QFR is strong quinone-binding at the O1 carbonyl that provides an axis for Q to rotate in and out of the Q-site within the same plane of the benzo- or napthoquinone rings similar to the motion of a pendulum as modeled in Fig. 3.

The X-ray structure for SQR shows no direct contact between the O4 carbonyl of UQ and the proposed H-donor Ser C27 [6]. Computational and structural studies have suggested that the position where UQ is bound may not be the actual site where UQ intermediates are stabilized during catalysis [29]. Mutagenesis and kinetic data have demonstrated the importance of Ser C27, Arg C31, and Asp D82 [38,41] and the computational data [29] modeled UQ deeper into the quinone-binding pocket so that it makes contact with Ser C27 and His B207 in what was proposed as the catalytic position. The model was confirmed structurally by the location of the potent quinone-site inhibitors atpenin A5 and carboxin [29,30] which make H-bond contact with the Ser C27 and His B207. As for the movement of the quinone described above for QFR the quinone moves to the catalytic site with a pendulum motion as the benzoquinone ring O1 carbonyl is anchored by Trp B164 and Tyr D83 that enables the swinging movement. The mechanism for UQ reduction by SQR, thus involves the binding of UQ at the entry of the Q-site (shown in green on the right side of Fig. 3), and upon reduction of the redox chain by succinate, UQ swings into its catalytic position (shown in blue on the right of Fig. 3). An enabling factor in this swinging motion appears to be through the 3-methoxy group of UQ which forms an H-bond to His B207 to possibly stabilize the ubisemiquinone radical [29,38]. These structural studies agree with previous studies showing that modification of the 2- or 3-methoxy groups of the benzoquinone ring affect the activity of bovine SQR [42]. In those studies there was a complete loss of activity when either methoxy group was substituted by a methyl group [42]. More recently it has been shown that the 2-methoxy group of ubiquinone is essential for the quinone acceptor function in reaction centers from *Rhodobacter sphaeroides* [43]. The reaction center was the first membrane protein for which primary and distal positions for quinones were observed at the Q_b-site [23,24]. When UQ is bound at the stand-by position it makes a single H-bond to the protein but upon formation of the semiquinone at the Q_a-site the quinone rotates and flips into the catalytic position where multiple H-bonds including many to methoxy groups are established [23,24,43]. Thus, it is worth suggesting that the methoxy substituents of the quinone head groups play an important role in catalysis in general. First, it is known that the dihedral angle of methoxy groups on benzoquinones can have an impact on the redox potential of the quinone and on catalytic activity with proteins [43–45]. Second, methoxy groups may be critical for movement into the catalytic position and/or for stabilization of the semiquinone intermediates during redox reactions [29,43]. Third, methoxy groups may be important for catalysis by participating in coordination of catalytic water(s) for H^+-exchange. The latter suggestion helps to explain the retention of significant catalytic activity in the *E. coli* SdhD Tyr83 mutants [38], in contrast to the yeast enzyme where different substitutions of the equivalent residue resulted in only a minimal retention of catalytic activity [39]. The conserved waters seen in the *E. coli*, avian, and pig SQR structures [6,7,15,30] are coordinated by several H-bonds from protein, as well as, from the 2-methoxy group of UQ. Thus, conserved water may play a role in catalysis as either a direct H^+-donor to the O1 oxygen atom of the benzoquinone in the native protein or substitute for Tyr D83 in the mutant enzymes. These findings are in agreement with a recent mutagenesis study of potential water coordinating amino acids in *E. coli* SQR [46].

Further characterization of the dynamics of quinone movement during catalysis is needed in order to fully define the quinone-binding site of complex II. Overall the studies of the quinone-binding site of both SQR and QFR show that there is significant interplay between the protein, the solvent, and the quinone itself in order for complex II to be proficient in catalysis.

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